

## CONCISE COMMUNICATION

# Increased Liver Decompensation Risk with Atypical Hepatitis C Virus Antibody Levels

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Knowledge of serum markers of liver decompensation would facilitate care of patients with hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections. HCV load and anti-c33c and anti-NS5 levels did not distinguish 28 HCV- and HIV-positive predecompensation patients from 28 matched control patients, whereas more patients than controls had high anti-c100(p) and low anti-c22(p). In multivariate analysis, decompensation was associated with high anti-c100(p) titer ( $\geq 1:4050$ ; odds ratio [OR], 3.4; 95% confidence interval [CI], 1.1–11.5) and low anti-c22(p) ( $< 1:36,450$ ; OR, 3.0; 95% CI, 1.0–10.2) and with antibody band strength at 1:50 dilution (anti-c100[p] OR, 7.0; 95% CI, 1.7–48.9; anti-c22[p] OR, 7.1; 95% CI, 1.7–49.2). With high anti-c100(p) or low anti-c22(p), sensitivity for decompensation was 86%–96% and specificity was 21%–36%; with both markers, sensitivity was 29%–32% and specificity was 93%–96%. Although the mechanisms for these associations are unknown, if these findings are verified in other populations, anti-c100(p) and anti-c22(p) might be valuable surrogate markers for liver decompensation risk.

Most people who acquired human immunodeficiency virus (HIV) infection through parenteral drug use or infusion of clotting factor concentrates to treat hemophilia were coinfecting with hepatitis C virus (HCV), and several reports indicate that coinfecting patients have a higher risk of cirrhosis and liver decompensation than do those infected only with HCV [1–4]. Because HIV infection is associated with cellular immune deficiency and perturbations of humoral immunity [5, 6], it is not surprising that antibody reactivity against HCV may be altered with HIV coinfection [7, 8]. We investigated the possibility that antibody responses against HCV might be predictive of liver decompensation among people with hemophilia who were coinfecting with HCV and HIV.

## Methods

**Subjects.** Among HIV-HCV-coinfecting hemophilic subjects enrolled in the Multicenter Hemophilia Cohort Study [9], we identified 28 subjects who had developed liver decompensation, which was defined as persistent ascites, bleeding esophageal varices, hepatic encephalopathy, or death, excluding nonhepatic causes [1]. We then selected from the hemophilia cohort 28 HIV-HCV-coinfecting control subjects who were frequency matched to the patients by geography (United States vs. Europe) and, at the time of serum collection, age ( $\pm 5$  years), duration of HIV infection, and CD4<sup>+</sup> lymphocyte count. The matching was evaluated with median and interquartile range (IQR) values.

**Virus load and antibody testing.** HCV load in the 28 patients and 28 controls was determined with branched DNA technology (Quantiplex HCV RNA 2.0 Assay [bDNA], Chiron, Emeryville, CA), with a lower limit of sensitivity of 200,000 (5.3 log<sub>10</sub>) genome equivalents/mL. The 56 coded sera also were tested for HCV antibody reactivity with the third-generation recombinant immunoblot assay (RIBA; HCV RIBA3.0, Chiron), at the baseline dilution recommended by the manufacturer (1:50) and at 6 additional 3-fold dilutions, ranging from 1:150 to 1:36,450. The intensity of each RIBA band was read against an 11-point external gray scale (pure white = 0, pure black = 10; figure 1, insert).

**Statistical analysis.** Repeated-measures analysis of variance (the *F* test) was used to examine differences among the reactivities, and the Spearman correlation coefficient was used to evaluate associations between reactivities at 1:50 and end-point titers (the last dilution with a band intensity  $\geq 2$ ). Univariate and multivariate logistic regression was used for the case-control comparison of the

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Informed consent was obtained from the patients or their parents or guardians, and human experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions were followed in the conduct of clinical research.

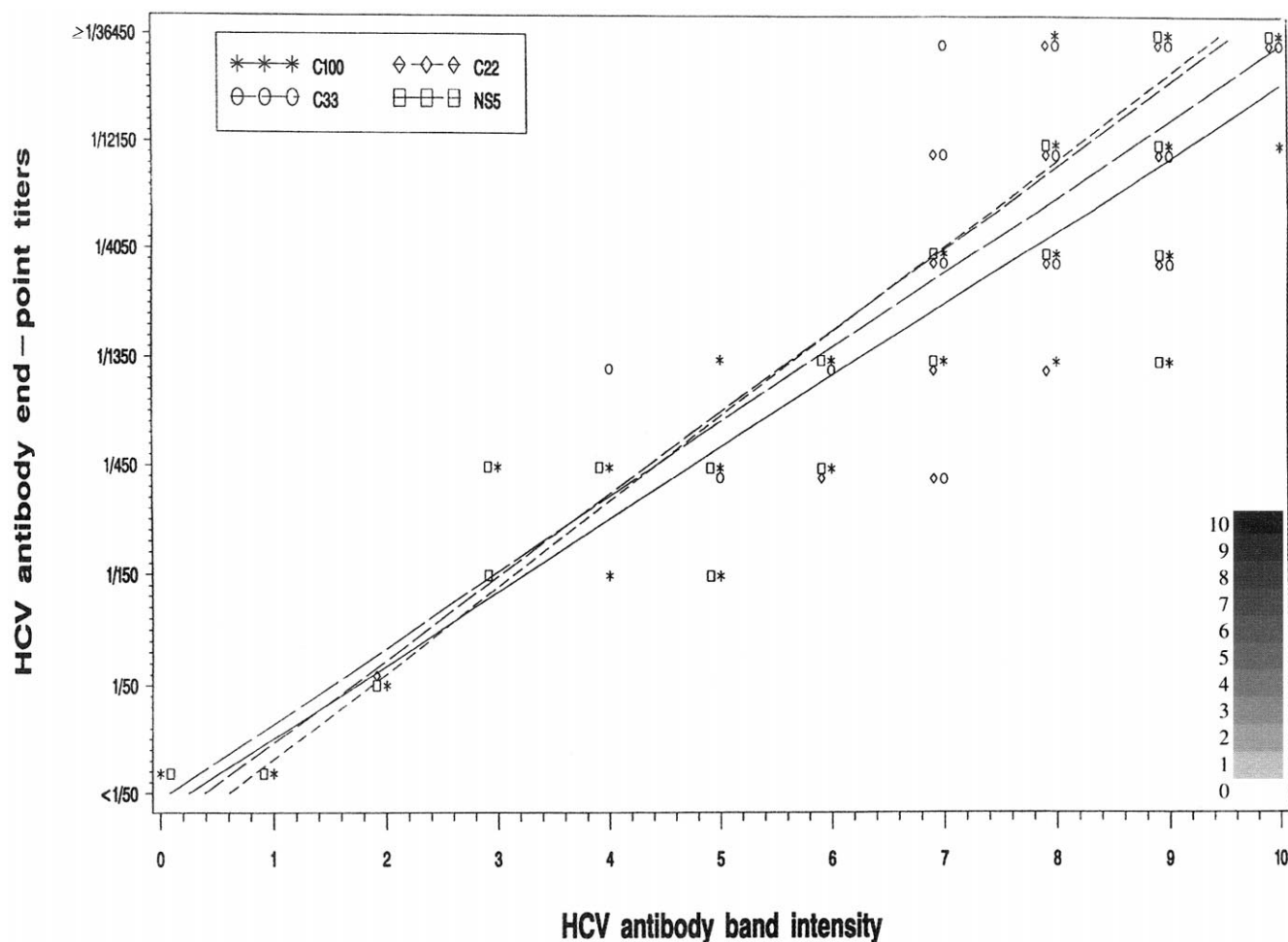
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**Figure 1.** Distribution of antibody reactivity against 4 hepatitis C virus (HCV) antigens, as detected by third-generation recombinant immunoblot assay (RIBA). In 56 hemophilic subjects, the intensity of the RIBA bands from pure white to pure black (0–10) at a serum dilution of 1:50 was measured by use of the scale shown in the *insert* (lower right). Band intensity was strongly correlated with the end-point titers from <1:50 to  $\geq 1:36,450$ . Regression lines for each of 4 antigens are shown (Spearman  $R = .62-.95$ ;  $P \leq .0001$  for each antigen).

risk of liver decompensation associated with HCV load ( $\log_{10}$  transformed), the antibody band intensity at 1:50, and the geometric mean end-point titers of antibodies. Median titer, band intensity, and virus load values were used to categorize subjects as high or low. The likelihood ratio test was used for retention of variables in the final multivariate model. Sensitivity (the proportion of positive patients) and specificity (the proportion of negative control patients) were calculated for assays individually and in combination.

## Results

**Virus load and antibody levels and correlations.** HCV load for the 56 subjects was in the expected range, with a median of 6.717 (IQR 6.0–7.3)  $\log_{10}$  genome equivalents/mL. For each of the 4 HCV RIBA antigens, the intensity of the antibody band at the 1:50 dilution and the end-point antibody titer

against the same antigen were highly correlated ( $R = .62-.95$ ;  $P \leq .0001$ ; figure 1). Antibody titers against NS5, c100(p), and c33c were highly correlated with one another ( $R = .61-.74$ ;  $P \leq .0001$ ), whereas titers against c22(p) were unrelated to titers against the other 3 antigens ( $R = .04-.19$ ;  $P > .15$ ). There was no correlation of virus load with antibody titers to any of the 4 antigens ( $R \geq .16$ ;  $P > .23$ ).

At the 1:50 dilution, the mean antibody band intensity against the NS5 antigen was only 5.2, which was significantly weaker than the antibody intensity against antigens c100(p) (mean, 6.5;  $P = .006$ ), c33c (mean, 8.1;  $P = .0001$ ), and c22(p) (mean, 8.2;  $P = .0001$ ). Likewise, the end-point antibody titer against NS5 was only 1:764, which was significantly lower than the titers against c100(p) (1:1611;  $P = .02$ ), c33c (1:12,150;  $P = .0001$ ), and c22(p) (1:12,391;  $P = .0001$ ).

**Case-control analysis.** At the time when the serum samples

**Table 1.** Recombinant immunoblot assay band strength and end-point titers in liver decompensation patients with hepatitis C virus (HCV) and human immunodeficiency virus coinfection and matched controls.

HCV assay	Patients with liver decompensation, no. (%) [ <i>n</i> = 28]	Matched controls, no. (%) [ <i>n</i> = 28]	Univariate <i>P</i> value	Odds ratio (95% CI) <sup>a</sup>
High- or low-band intensity and HCV load				
c100(p) band $\geq 8$	17 (61)	11 (39)	.11	7.0 (1.7–48.8)
c22(p) band $< 9$	18 (64)	12 (43)	.11	7.1 (1.7–49.2)
c33c band $\geq 9$	19 (68)	19 (68)	1.00	NS
NS5 band $\geq 6$	16 (57)	14 (50)	.59	NS
HCV load $\geq 6.717 \log_{10}$	17 (61)	11 (39)	.11	NS
High or low titer and HCV load				
c100(p) titer $\geq 1:4050$	17 (61)	10 (36)	.06	3.4 (1.1–11.5)
c22(p) titer $< 1:36,450$	16 (57)	10 (36)	.11	3.0 (1.0–10.2)
c33c titer $\geq 1:36,450$	16 (57)	14 (50)	.59	NS
NS5 titer $\geq 1:4050$	16 (57)	13 (46)	.42	NS
HCV load $\geq 6.717 \log_{10}$	17 (61)	11 (39)	.11	NS

NOTE. CI, confidence interval; NS, not significant.

<sup>a</sup> Multivariate adjusted odds ratios as measures of relative risk for above versus below median values for band intensity, from 0 (white) to 10 (black), at 1:50 dilution; for antibody end-point titer; and for virus load ( $\log_{10}$  genome equivalents/mL).

were collected, the patients and controls were similar in age (median 28 [IQR 16–37] years vs. 25 [IQR 15–37] years, respectively), in CD4<sup>+</sup> lymphocyte count (median 249 [IQR 146–526] cells/ $\mu$ L vs. 315 [IQR 153–566] cells/ $\mu$ L, respectively), and in duration of HIV infection (median 79 [IQR 64–95] months vs. 81 [IQR 61–92] months, respectively). Liver decompensation developed in the patients a median of 31 (IQR 12–69) months later. Patients with liver decompensation and matched controls had broadly similar HCV loads, antibody band intensities at the 1:50 dilution, and end-point antibody titers, although reactivity against c100(p) tended to be higher among patients than among controls. The anti-c100(p) band intensity in patients was 7.1, versus 5.8 in controls (*P* = .08), and the corresponding end-point titers were 1:2845 in patients versus 1:912 in controls (*P* = .06).

By use of median values, we found that neither band intensity nor titers against c33c or NS5 distinguished patients from control individuals (table 1). However, borderline significant differences were noted with high anti-c100(p) band intensity ( $\geq 8$ ; 61% of patients) and titer ( $\geq 1:4050$ ; 61% of patients), low anti-c22(p) band intensity ( $< 9$ ; 64% of patients) and titer ( $< 1:36,450$ ; 57% of patients), and high HCV load ( $\geq 6.717 \log_{10}$  genome equivalents/mL; 61% of patients). Virus load did not contribute significantly to multivariate models. In contrast, high anti-c100(p) and low anti-c22(p) levels were strongly and independently related to risk, with odds ratios (ORs) of 7.0–7.1 (95% confidence interval [CI], 1.7–49.2) for band intensity and ORs of 3.0–3.4 (95% CI, 1.0–11.5) for titer (table 1).

By use of band intensity or titer, we found that each assay alone had 57%–64% sensitivity and specificity. With 2 assays, sensitivity was 96% and specificity was 21% with high anti-c100(p) or low anti-c22(p) band intensity. Corresponding sensitivity and specificity by use of titer were 86% and 36%,

respectively, with high anti-c100(p) or low anti-c22(p) titer. With both high anti-c100(p) and low anti-c22(p) band intensity, sensitivity was 29% and specificity was 96%. With titers, corresponding sensitivity and specificity were 32% and 93%, respectively, with both high anti-c100(p) and low anti-c22(p) titers.

## Discussion

Several studies have noted that the majority of patients with HIV coinfection lack or lose HCV antibody reactivity against c100(p), c5-1-1, c33c, and NS5 antigens but generally not against c22(p) antigens [7, 8, 10]. We postulated that antibody reactivity might reflect the activity of liver disease or the risk of liver decompensation. In HIV-coinfected subjects who did or did not progress to liver decompensation during an average of 3 years of follow-up, we found similar mean levels of HCV load and antibody reactivity against 3 of 4 HCV antigens. Patients who progressed to liver failure had an ~3-fold (1 dilution) higher titer of anti-c100(p), a finding of marginal statistical significance. Categorization based on median values and logistic regression to adjust for the level of each antibody was more revealing. When end-point titers were used, the risk of liver decompensation was significantly increased 3.4-fold with high anti-c100(p) titer ( $\geq 1:4050$ ) and 3-fold with low anti-c22(p) titer ( $< 1:36,450$ ). With the simpler and less expensive measure of antibody intensity from 0 to 10 at the conventional 1:50 serum dilution, which was highly correlated with end-point titer, the risk of liver decompensation was significantly increased 7-fold with high anti-c100(p) and 7.1-fold with low anti-c22(p). HCV load was slightly, but not significantly, higher in patients with liver decompensation. Because we had not postulated these findings a priori, they must be corroborated, both in HIV-HCV-coinfected subjects and in those infected only with HCV.

Hepatic inflammation and subsequent fibrosis appear to result from an inability to eradicate HCV-infected hepatocytes, a process that probably hinges on a potent Th1-type cytokine response derived from CD4<sup>+</sup> cell-mediated immunity [11]. We postulate that antibody reactivity associated with liver decompensation probably reflects a dysfunctional Th2-type cellular immune response [12, 13], resulting in the elaboration of cytotoxic T cells or cytokines, which leads to hepatic inflammation and fibrosis. The c100(p) peptide (aa 1920–1935 of the NS4 gene) overlaps an HCV peptide (aa 1909–1929) that contains an immunodominant epitope “capable of binding to multiple human leukocyte antigen alleles and of being recognized by T cells in a promiscuous manner” [14, page 1094]. As others have noted in HIV-HCV-coinfected patients [7, 8, 10], we found that anti-c22(p) levels were not correlated with antibody levels against the other HCV epitopes. Because anti-c22(p) levels usually are preserved, low anti-c22(p) reactivity is atypical and, on the basis of our findings, is associated with liver decompensation. Determination of whether the risk associated with this atypical pattern reflects a dysfunctional cellular immune response, some other mechanism, or merely chance will require additional research.

We found that atypical (high c100[p], low c[22]p) antibody levels had 57%–64% sensitivity and specificity when used singly. Only 2 (7.1%) of control subjects had atypical titers to both antigens, and only 1 (3.6%) had atypical bands to both antigens—that is, a specificity of 93%–96%. Conversely, of the 28 subjects who progressed to liver decompensation, 24 (86%) had atypical titers, and 27 (96%) had atypical bands to at least 1 of the 2 antigens.

Surrogate markers of increased risk of liver decompensation might be of value in the assessment and management of HCV-infected patients with hemophilia, for whom there is considerable risk associated with liver biopsy and considerable cost for clotting factor replacement to mitigate hemorrhage [15]. The potential benefits of a noninvasive marker are magnified in the setting of HIV coinfection, because of the higher risk of cirrhosis and liver decompensation and the unknown efficacy, adverse effects, and drug interactions of combination anti-HCV therapy. Efforts should be made both to validate our observation that high c100(p) and low c(22)p antibody levels are associated with liver decompensation and to examine other potential surrogate markers of progressive liver disease.

#### **Institutions and Investigators in the Multicenter Hemophilia Cohort Study**

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